the intervening expression cassette comprising a plant promoter operably linked to a polynucleotide encoding a second polypeptide;

wherein at least the first or the second plant promoter is a non-constitutive promoter; wherein the first and second polypeptides each comprise a separate but complementary amino acid subsequence of a single functional nuclease polypeptide; and wherein expression of the first and second polypeptides in the same cell results in production of a functional nuclease polypeptide, which thereby impairs cellular function.

REMARKS

The Present Invention

The present invention provides a two-component system to produce, *e.g.*, a lethal effect in plant cells. In this system, two polypeptides are expressed in a plant cell. The polypeptides are encoded by expression cassettes located at the same locus on each of two homologous chromosomes. One expression cassette comprises a first promoter operably linked to a first polynucleotide sequence, with a recombinase site between the first promoter and the first polynucleotide sequence. The second expression cassette comprises the first plant promoter inoperably linked to the first polynucleotide due to the presence of an intervening expression cassette, flanked by two recombinase sites, between the first promoter and the first polynucleotide. The intervening expression cassette comprises a second promoter operably linked to a second polynucleotide.

Each expression cassette of the invention is individually functional, but the product of each cassette alone does not provide the desired lethal effect. The combination of the two polypeptides from the individual expression cassettes is required for producing the lethal effect. The first and second polypeptides are separate but complementary amino acid subsequences of a single functional nuclease. Expression of the first and second polypeptides in the same cells results in production of a functional nuclease polypeptide, thereby impairing cellular function.

Status of the Claims

Claims 1-4, 6, 7, 11-18, 20, 21, and 25-37 are pending in the above-referenced patent application. Claims 3, 13, 17, and 27 have been withdrawn without prejudice to future prosecution. Claims 1 and 14 have been amended.

In particular, claims 1 and 14 have been amended to recite that "the first and second polypeptides each comprise separate but complementary amino acid subsequences of a single functional nuclease" and that "expression of both the first and second polypeptides in the same cell results in production of a functional nuclease polypeptide." These amendments are supported at page 11, lines 21-31 and page 13, lines 18 to page 14, line 3 of the specification. Thus, no new matter is added by the amendments.

In addition, claim 14 has been amended to recite "a method of impairing cellular function in a plant." This amendment is supported at page 4, line 23 to page 5, line 2 and page 14, lines 15-19. Thus, no new matter is added by the amendment.

The amendments to claims 1 and 14 were made ***solely*** to clarify the claimed subject matter. Thus, the amendments add no new matter.

For the convenience of the Examiner, a marked-up version of the changes made to the claims by the present Amendment is attached as Appendix A. In addition, all of the pending claims are attached as Appendix B.

In the present Office Action, the pending claims were rejected, in various combinations, under 35 U.S.C. § 112, second paragraph, under 35 U.S.C. § 112, first paragraph, and under 35 U.S.C. § 103(a). Each of these rejections is addressed below.

Rejection under 35 U.S.C. § 112, second paragraph: definiteness

Claims 1-4, 6, 7, 11-18, 20, 21, and 25-37 were rejected as allegedly failing to particularly point out and distinctly claim the subject matter the Applicant regards as their invention.

According to MPEP § 2173.02, definiteness of claim language must be analyzed in light of: "(A) the content of the particular application disclosure; (B) the

teachings of the prior art; and (C) the claim interpretation that would be given by one of ordinary skill in the art at the time the invention was made."

With regard to claim 1, the rejection states that the claim lacks a recitation that the subsequences are complementary and when used together make the whole functional nuclease. Office Action, page 1, lines 18-19. Claim 1 has been amended in accordance with the Examiner's suggestion to recite that "the first and second polypeptides each comprise separate but complementary amino acid subsequences of a single functional nuclease" and that "expression of both the first and second polypeptides in the same cell results in production of a functional nuclease."

With regard to claim 14, the rejection states that the recitation of "a method of modifying cellular function is indefinite." Office Action, page 2, lines 1-2. Claim 14 has been amended to recite "a method of impairing cellular function." The specification contains specific guidance as to what constitutes impairment of cellular function. For example, the specification at page 4, line 23 to page 5, line 2 describe impairment of cellular function as, *e.g.*, killing cells or inhibiting cell division or cell differentiation.

With regard to claims 29 and 34, the rejection states that the recitation of "overlapping specificities" is unclear. Applicants respectfully assert that one of skill in the art would understand from reading the specification that overlapping specificities, when used in reference to promoters, refers to patterns of tissue expression of the promoters. First, the specification at page 6, lines 3-11 explicitly describes tissue specific promoters. Second, the specification at page 9, lines 24-29 explicitly describes methods of identifying promoters with tissue specific expression. Finally, the specification at page 11, lines 1-4 explicitly states that two promoters can be used in the present invention and that the two promoters may be promoters whose tissue expression pattern overlaps. Thus, based on the guidance in the specification, one of skill in the art would understand that the recitation of "overlapping specificities" refers to functional and physical overlap between two promoters.

In view of the foregoing, Applicants respectfully submit that the claims ***do*** particularly point out and distinctly claim the subject matter the Applicant regards as their invention and request withdrawal of the rejection under 35 U.S.C. § 112, second paragraph.

Rejection under 35 U.S.C. § 112, first paragraph: enablement

Claims 1-4, 6, 7, 11-18, 20, 21, and 25-37 were rejected as allegedly lacking enablement. Applicants respectfully traverse.

The proper test of enablement is "whether one skilled in the art could make or use the claimed invention from the disclosure in the patent coupled with information known in the art without undue experimentation." *See, e.g.*, MPEP § 2164.01. As identified by the Patent Office and the Federal Circuit, whether undue experimentation is required by one skilled in the art to practice the invention is determined by considering factors such as the breadth of the claims, the level of one of ordinary skill, amount of guidance presented in the application, and the presence of working examples. *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int. 1985); *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). As described in *Wands*, "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should precede." *Wands*, 8 USPQ2d at 1404 (quoting *In re Jackson*, 217 USPQ 804 (Bd. Pat. App. & Int. 1982).

The rejection states generally that in view of the breadth of the claims, the lack of guidance in the specification, and the unpredictability in the recombinase art, undue experimentation would be required for one of skill in the art to practice the claimed invention.

Applicants first note that the claims have been amended to recite plant cells comprising two expression cassettes comprising polynucleotide sequences each encoding separate ***but complementary*** amino acid subsequences of a single functional nuclease. The amended claims further recite that expression of the separate but

complementary amino acid sequences leads to production of a functional nuclease polypeptide, thereby impairing cellular function. However, to the extent that the rejection remains applicable to the claims as amended, Applicants respectfully traverse. Methods of transforming plant cells are well known in the art, and the specification provides guidance for making expression vectors that express the nucleases of the invention, and selecting promoters for expression of the nucleases in plants. Moreover, Applicants have previously presented evidence that the claimed methods can be used to make transgenic plants wherein the desired lethal effect is produced in cells. Thus, one of skill in the art would be able to practice the claimed invention with, at most, only routine experimentation.

Complementary amino acid subsequences produce a functional nuclease polypeptide when expressed in the same cell

First, the rejection states that the claims lack a recitation that the polynucleotide subsequences need to be complementary and when used together make the whole functional nuclease. Office Action page 3, lines 15-17. Claims 1 and 14 have been amended as suggested by the Examiner to recite that "the first and second polypeptides each comprise separate but complementary amino acid subsequences of a single functional nuclease" and that "expression of both the first and second polypeptides in the same cell results in production of a functional nuclease polypeptide."

Use of a nuclease to produce the lethal effect is predictable

The rejection also states that generation of a particular phenotype by introducing transgenes into plant cells is unpredictable. Office Action, page 3, lines 18-19. Applicants respectfully note that the claims have been amended to recite that the desired lethal phenotype is achieved by production of a functional ***nuclease*** polypeptide by expression of the two separate ***but complementary*** amino acid subsequences of the functional nuclease in the same cell. Nucleases have a known mechanism of action, so their effect in the plant cell is predictable: RNA or DNA essential for cell survival is degraded.

Applicants respectfully submit that one of skill in the art would be able to make and distinguish operative nuclease embodiments using the guidance in the specification. For example, the specification at pages 15-17 teaches how to make and use two-non-functional nuclease subsequences that together make a single functional nuclease, *e.g.*, using partial proteolysis, sequence conservation-based design, or structure based design. The specification also provides an example where the ribonuclease barnase has been cleaved into two subsequences which together form a functional enzyme (*see, e.g.*, specification page 17). Finally, assays for nuclease cytotoxicity are discussed at page 17 of the specification.

Applicants have also demonstrated that the claimed methods can be used to make transgenic plants wherein the desired lethal effect is produced in plants expressing a functional nuclease. For example, the declaration of Dr. Neal Gutterson submitted on November 17, 2002 with the response to the Office Action mailed May 18, 2000 provides experimental results demonstrating that the desired lethal effect is produced in transgenic plants. Specifically, transgenic plants expressing two separate but complementary subsequences of a single functional protein (*e.g.*, a nuclease, barnase) under the control of a tapetal specific promoter showed impaired cellular function in the cells in which the polypeptide subsequences were expressed. Thus, Applicants have ***actually*** demonstrated that the desired lethal effect of a functional nuclease polypeptide produced from two separate but complementary subsequences is produced in transgenic plants. In view of the explicit guidance in the specification and the experimental data presented by the Applicants, it is respectfully asserted that the present invention is fully enabled.

Identification of impaired cellular function is routine

The rejection further states that the claims drawn to modifying cellular functions are not enabled because there are many cellular functions and thus, potentially infinite numbers of modifications. Office Action, page 4, lines 11-14. Claim 14 has been amended to recite "***impairing*** cellular functions in a plant" (emphasis added) by

expressing polypeptides that comprise separate but complementary sequences of a functional nuclease, wherein production of the functional nuclease polypeptide impairs cellular function. Applicants respectfully submit that the specification provides ample guidance for one of skill in the art to practice the claimed method of ***impairing*** cellular function in a plant. For example, the specification at page 4, line 23 to page 5, line 2 describes impairment of cellular function as, *e.g.*, disruption of a cell through perturbation of some function of the cell or by degradation of a component of the cell; or prevention of continued growth of the cell. Thus, explicit guidance regarding ***impairment*** of cellular function is provided in the specification. In addition, the specification at page 17, lines 14-29 describes assaying plant cell cytotoxicity or inhibition caused by transgenes. Thus, assays to identify impairment of cellular function are explicitly provided. Moreover, as explained above, Applicants have also demonstrated that the claimed methods can be used to make transgenic plants wherein the desired lethal effect, *i.e.*, ***impairment*** of cellular function, is produced in plants expressing a functional ***nuclease***. In conclusion, the claims are enabled because a person of ordinary skill in the art can routinely identify ***impairment*** of cellular function without undue experimentation by using the explicit guidance in the specification.

Selection of genes, promoters, and cassettes is routine

The rejection also states that Applicants do not provide guidance for which of the genes, promoters, and cassettes can be used with recombinase to successfully modify cellular function. Office Action, page 5, lines 7-10. As noted above, the claims have been amended to recite ***impairment*** cellular function by production of a functional ***nuclease***. Applicants respectfully assert that one of skill in the art would be able to routinely identify nucleases that impair cellular functions by using the explicit guidance in the specification. For example, the specification at page 14, line 29 to page 17, line 13 describes identification of suitable nuclease subsequences that can be used in the present invention. The specification also provides explicit guidance as to the combinations of promoters and expression cassettes that can be used in the present

invention. For example, identification of multiple promoters and combinations of promoters, including tissue-specific and constitutive promoters, useful with the present invention is described at page 9, line 22 to page 12, line 18. Likewise, expression cassettes suitable for use in the present invention are defined at page 4, line 16-22 of the specification. Moreover, as explained above, the specification provides explicit guidance as to what constitutes ***impaired*** cellular function (*see, e.g.*, page 4, line 23 to page 5, line 2).

Applicants have also demonstrated that two separate but complementary subsequences of the nuclease, barnase, produce the desired lethal effect in the cells of transgenic plants when the subsequences are expressed under the control of tissue specific promoters or constitutive promoters (*see*, declaration of Dr. Neal Gutterson). Thus, selection of genes, promoters, and cassettes for use in the present invention can routinely be accomplished by one of skill in the art using the explicit guidance in the specification.

Applicants also note that the Examiner's citation of Kilby *et al.*, a reference that allegedly describes a recombinase system *i.e.*, FLP/frt, as part of a 35 U.S.C. § 103(a) rejection (addressed below) is inconsistent with an allegation that explicit descriptions of particular recombinase systems in the specification do not enable one of skill in the art to practice the claimed invention. If a reference (*i.e.*, Kilby *et al.*) that describes a single recombinase system is enabling when cited as part of an obviousness rejection, an even more detailed disclosure describing multiple recombinase systems (*see, e.g.*, page 7, lines 1-5 of the specification) should also be enabling.

Identification of inoperable embodiments is routine

Finally the rejection states that the art is unpredictable and it would require undue experimentation to eliminate inoperable embodiments, *i.e.*, to identify the phenotype desired. Office Action, page 6, lines 16-19. As noted above, the claims have been amended to recite ***impairment*** of cellular function by production of a functional ***nuclease***. As explained above, Applicants respectfully assert that a skilled artisan, using

the guidance in the specification would routinely be able to identify inoperable embodiments of transgenic plants in which cellular function is not impaired.

Applicants respectfully submit that transformation of plant species is routine methodology well known to those of skill in the art. The specification at page 8, line 23 to page 9, line 23 explicitly describes multiple methods of plant transformation. Moreover, as explained above, Applicants have also demonstrated that the claimed methods can be used to make transgenic plants wherein the desired lethal effect is produced in plants expressing a functional nuclease (*see, e.g.*, declaration of Dr. Neal Gutterson). Specifically, transgenic plants expressing two separate but complementary subsequences of a single functional barnase under the control of a tapetal specific promoter showed impaired cellular function in the cells in which both of the polypeptide subsequences were expressed. Thus, Applicants have ***actually*** demonstrated that the cellular function is impaired when a functional nuclease polypeptide produced from two separate but complementary subsequences is produced in transgenic plants. In view of the explicit guidance in the specification and the experimental data presented by the Applicants, it is respectfully asserted that the present invention is fully enabled.

In view of the foregoing, Applicants submit that transformation of a plant with expression cassettes encoding a nuclease, wherein expression of the nuclease is lethal to the plant cell, requires only routine experimentation. Thus, the claimed invention is enabled and Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

Rejection under 35 U.S.C. § 103

Claims 1-4, 6, 7, 11-18, 20, 21, and 25-37 were rejected as allegedly unpatentable over Kilby *et al.* (1995) *Plant J.* 8(5):637, in view of Mariani *et al.* (1990) *Nature* 347:737, Sancho and Fersht (1992) *J. Mol. Bio.* 224:741, and Applicants' own admission. Applicants respectfully traverse.

> As set forth in M.P.E.P. § 2143, "[t]o establish a *prima facie* case of obviousness, *three* basic criteria must be met. *First*, there must be some suggestion or motivation, either

> in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *Second*, there must be a reasonable expectation of success. *Finally*, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

All three elements set forth above must be present in order to establish a *prima facie* case of obviousness. Moreover, according to the Federal Circuit, there are three sources of motivations to combine references: "the nature of the problem to be solved, the teachings of the prior art, and the knowledge of persons of ordinary skill in the art." *In re Rouffet,* 47 USPQ2d 1453, 1458 (Fed. Cir. 1998). To prevent the improper use of hindsight, the Federal Circuit has required a showing of motivation to combine references that create a case of obviousness. *Id.* at 1457-58. If "the specific understanding or principle within the knowledge of a skilled artisan that would motivate one with no knowledge of the invention" is not explained, it is inferred that the references were selected and combined with improper hindsight. *Id.* at 1458. Applicants respectfully assert that a *prima facie* case of obviousness has not been established for at least the following reason: one of ordinary skill in the art would not have been motivated to combine the cited references.

Applicants respectfully note that the claims have been amended to recite plant cells comprising two expression cassettes comprising polynucleotide sequences each encoding separate ***but complementary*** amino acid subsequences of a single functional nuclease. The amended claims further recite that expression of the separate but complementary amino acid sequences leads to production of a functional nuclease polypeptide, thereby impairing cellular function.. However, to the extent the rejection remains applicable to the claims as amended, Applicants respectfully traverse.

Kilby *et al.* is cited as teaching introducing into a plant a first and a second expression cassette, as claimed in the present invention. In particular, Kilby *et al.* is cited as teaching the use of intervening sequences flanked by recombination target sites within an expression cassette. Office Action page 8, lines 1-11. The rejection acknowledges that Kilby does not teach a plant promoter in the intervening cassette, a nuclease, lox sites, transactivator proteins, barnase, tissue specific promoters, seed coat or tapetal-specific promoters, but alleges that it would have been obvious to modify Kilby *et al.*, to insert ribonuclease genes controlled by tapetal-specific regulatory sequences; to modify Kilby to insert two amino acid subsequences of a single nuclease; or to substitute the Cre/lox system for the FLP/frt system of Kilby *et al.* Office Action page 9, lines 2-5. Mariani is cited as teaching ribonuclease genes controlled by tapetal specific sequences. Office Action page 8, lines 14-15. Sancho is cited as teaching a first and a second amino acid subsequence of a single functional nuclease. Office Action, page 8, lines 16-17.

As explained above, a *prima facie* case of obviousness requires some suggestion in the references themselves that would motivate a skilled artisan to combine the references. The rejection points to a statement in Mariani that "the ability of the TA29-RNase gene to induce male sterility provides a new strategy for production of crop plants" as motivation for combining the disclosures of all the cited references. However, Mariani goes on to state that the new strategy involves coupling the chimeric TA29-RNase gene to dominant herbicide genes, ***not*** that the chimeric gene may be used in conjunction with a recombinase expression cassette system. The rejection points to no other motivation in any of the cited references or the specification that would lead a skilled artisan to combine the disclosures of the references.

A perusal of each of the cited references and the specification reveals that no such motivation can be found. For example, Kilby *et al.* describe a method for generating marked sectors in *Arabidopsis* using an FLP/frt recombinase system. According to Kilby *et al.*, the system is particularly useful for activating the marker β-glucuronidase in transformed plant cells and may be used for analysis of gene function in chimeric plants (*see, e.g.*, page 638, column 2, lines 29-39). Kilby *et al.*, however, does

not mention or suggest that the described method may be used to produce a single functional nuclease in a plant wherein the presence of the nuclease is lethal to the plant. Likewise, Kilby *et al.* is devoid of any suggestion or mention that the described method can be used to impair cellular function in a plant. Mariani *et al.* describes a ***single*** component system in which the TA29 gene regulatory fragment is fused to a single sequence of a nuclease gene. There is no suggestion or mention in Mariani *et al.* of a two component system wherein a single functional nuclease polypeptide is produced when ***two*** separate but complementary amino acid subsequences of the same nuclease are expressed in the same cell. Sancho *et al.* describes a method for producing fragments of a protein suitable for studies of protein folding using the nuclease, barnase, as a model protein. Sancho *et al.* is ***wholly*** focused on studying the kinetics and thermodynamics of protein folding. There is no suggestion or mention in Sancho *et al.* that there is ***any*** other use for the described method of producing fragments of a protein. With regard to the statement in the specification that the multiple recombinase systems may be used with the present invention, there is still no explanation of the principle that would motivate a skilled artisan with no knowledge of the invention to combine the cited references.

Thus, only the application of improper hindsight would lead to a skilled artisan to combine Kilby *et al.*, Mariani *et al.*, and Sancho *et al.* with the statement in the specification. In the absence of any mention or suggestion that the methods may be combines, the skilled artisan would not be motivated to make such a combination.

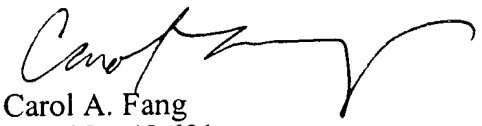
Accordingly, Applicants urge the Examiner to withdraw this rejection under 35 U.S.C. § 103(a).

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



Carol A. Fang
Reg. No. 48,631

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
CAF
SF 1354367 v1

APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (thrice amended) A plant containing a plant cell comprising a first and a second expression cassette located at the same locus on each of two homologous chromosomes, wherein:

the first expression cassette present on a first chromosome homolog comprises a first plant promoter operably linked to a first polynucleotide sequence encoding a first polypeptide, wherein a recombinase site is present between the first promoter and the first polynucleotide sequence;

the second expression cassette present on a second chromosome homolog comprises the first plant promoter inoperably linked to the first polynucleotide sequence, wherein an intervening expression cassette is flanked by two recombinase sites and situated between the first promoter and the first polynucleotide sequence of the second expression cassette, the intervening expression cassette comprising a second plant promoter operably linked to a second polynucleotide sequence encoding a second polypeptide;

wherein at least the first or the second plant promoter is a non-constitutive promoter; [wherein at least the first or the second polynucleotide encodes an amino acid sequence of a nuclease or] wherein the first and second [polynucleotides each encode] polypeptides each comprise a separate but complementary amino acid subsequence of a single functional nuclease wherein expression of both the first and second polypeptides in the same cell results in production of a functional nuclease polypeptide, thereby impairing cellular function[; and

wherein the presence of the first and second polypeptides in a cell is lethal to the cell].

[3. (as filed) The plant of claim 1, wherein the first polypeptide is a transactivator protein.]

[13. (previously once amended) The plant of claim 1, wherein the first and second polypeptides each comprise a separate subsequence of a single functional nuclease polypeptide.]

14. (thrice amended) A method of [modifying] impairing cellular function in a plant, the method comprising the steps of:

introducing into a plant a first expression cassette comprising a first plant promoter operably linked to a first polynucleotide encoding a first polypeptide, wherein a recombinase site is present between the first promoter and the first polynucleotide;

introducing into the plant a second expression cassette comprising the first plant promoter inoperably linked to a polynucleotide encoding the first polypeptide, wherein an intervening expression cassette is flanked by recombinase sites and situated between the first promoter and the first [polypeptide] polynucleotide of the second expression cassette, the intervening expression cassette comprising a plant promoter operably linked to a polynucleotide encoding a second polypeptide;

wherein at least the first or the second plant promoter is a non-constitutive promoter; [wherein at least the first or the second polynucleotide encodes an amino acid sequence of a nuclease or] wherein the first and second [polynucleotides each encode] polypeptides each comprise a separate but complementary amino acid subsequence of a single functional nuclease polypeptide; and wherein expression of the first and second polypeptides in the same cell results in production of a functional nuclease polypeptide, which thereby impairs cellular function[; and

wherein the presence of the first and second polypeptides in a cell is lethal to the cell].

[17. (as filed) The method of claim 14, wherein the first polypeptide is a transactivator protein.]

[27. (previously once amended) The method of claim 14, wherein the first and second polypeptides each comprise a separate subsequence of a single functional nuclease polypeptide.]

APPENDIX B

PENDING CLAIMS

1. (thrice amended) A plant containing a plant cell comprising a first and a second expression cassette located at the same locus on each of two homologous chromosomes, wherein:

the first expression cassette present on a first chromosome homolog comprises a first plant promoter operably linked to a first polynucleotide sequence encoding a first polypeptide, wherein a recombinase site is present between the first promoter and the first polynucleotide sequence;

the second expression cassette present on a second chromosome homolog comprises the first plant promoter inoperably linked to the first polynucleotide sequence, wherein an intervening expression cassette is flanked by two recombinase sites and situated between the first promoter and the first polynucleotide sequence of the second expression cassette, the intervening expression cassette comprising a second plant promoter operably linked to a second polynucleotide sequence encoding a second polypeptide;

wherein at least the first or the second plant promoter is a non-constitutive promoter; wherein the first and second polypeptides each comprise a separate but complementary amino acid subsequence of a single functional nuclease wherein expression of both the first and second polypeptides in the same cell results in production of a functional nuclease polypeptide, thereby impairing cellular function.

2. (as filed) The plant of claim 1, wherein the recombinase sites are lox sites.

4. (as filed) The plant of claim 1, wherein the intervening expression cassette is in reverse orientation with respect to the second expression cassette.

6. (previously twice amended) The plant of claim 1, wherein at least the first or the second polynucleotide encodes an amino acid sequence of a ribonuclease or wherein the first and second polynucleotides each encode a separate amino acid subsequence of a single functional ribonuclease.

7. (as filed) The plant of claim 6, wherein the ribonuclease is Barnase.

11. (as filed) The plant of claim 1, wherein the first or the second promoter is a tissue-specific promoter.

12. (as filed) The plant of claim 1, wherein the first and second promoters are each functional in tapetal cells.

14. (thrice amended) A method of impairing cellular function in a plant, the method comprising the steps of:

introducing into a plant a first expression cassette comprising a first plant promoter operably linked to a first polynucleotide encoding a first polypeptide, wherein a recombinase site is present between the first promoter and the first polynucleotide;

introducing into the plant a second expression cassette comprising the first plant promoter inoperably linked to a polynucleotide encoding the first polypeptide, wherein an intervening expression cassette is flanked by recombinase sites and situated between the first promoter and the first polynucleotide of the second expression cassette, the intervening expression cassette comprising a plant promoter operably linked to a polynucleotide encoding a second polypeptide;

wherein at least the first or the second plant promoter is a non-constitutive promoter; wherein the first and second polypeptides each comprise a separate but complementary amino acid subsequence of a single functional nuclease polypeptide; and wherein expression of the first and second polypeptides in the same cell results in production of a functional nuclease polypeptide, which thereby impairs cellular function.

15. (as filed) The method of claim 14, wherein the two expression cassettes are introduced through a sexual cross and the two expression cassettes are present on chromosome homologs.

16. (as filed) The method of claim 14, wherein the recombinase sites are lox sites.

18. (as filed) The method claim 14, wherein the intervening expression cassette is in reverse orientation with respect to the second expression cassette.

20. (previously twice amended) The method of claim 14, wherein at least the first or the second polynucleotide encodes an amino acid sequence of a ribonuclease or wherein the first and second polynucleotides each encode a separate amino acid subsequence of a single functional ribonuclease.

21. (as filed) The method of claim 20, wherein the ribonuclease is Barnase.

25. (as filed) The method of claim 14, wherein the first or the second promoter is a tissue-specific promoter.

26. (as filed) The method of claim 14, wherein the first and second promoters are each functional in tapetal cells.

28. (as filed) The plant of claim 1, wherein both the first and the second promoters are non-constitutive promoters.

29. (as filed) The plant of claim 1, wherein the first and second promoters have overlapping specificities.

30. (as filed) The plant of claim 1, wherein the first or the second promoter is a seed coat-specific promoter.

31. (as filed) The plant of claim 6, wherein the ribonuclease is ribonuclease T1 or binase.

32. (as filed) The plant of claim 6, wherein the first and second polypeptides each comprise a separate subsequence of a single functional ribonuclease polypeptide.

33. (as filed) The plant of claim 14, wherein both the first and the second promoters are non-constitutive promotes.

34. (as filed) The method of claim 14, wherein the first and second promoters have overlapping specificities.

35. (as filed) The method of claim 14, wherein the first or the second promoter is a seed coat-specific promoter.

36. (as filed) The method of claim 20, wherein the ribonuclease is ribonuclease T1 or binase.

37. (as filed) The method of claim 20, wherein the first and second polypeptides each comprise a separate subsequence of a single functional ribonuclease polypeptide.